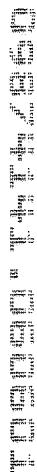
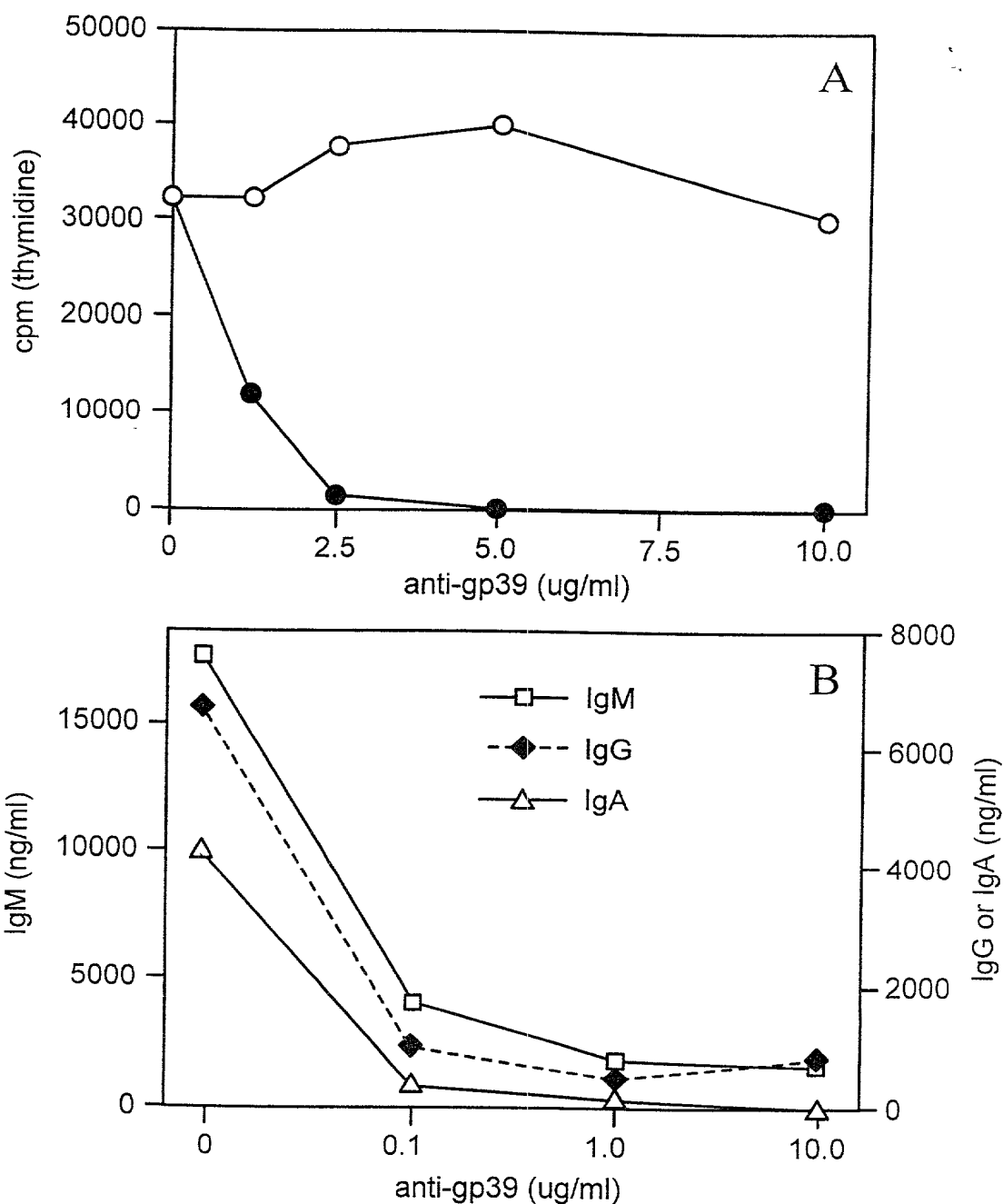


[illegible][illegible]

BLAC = Betalactamase gene

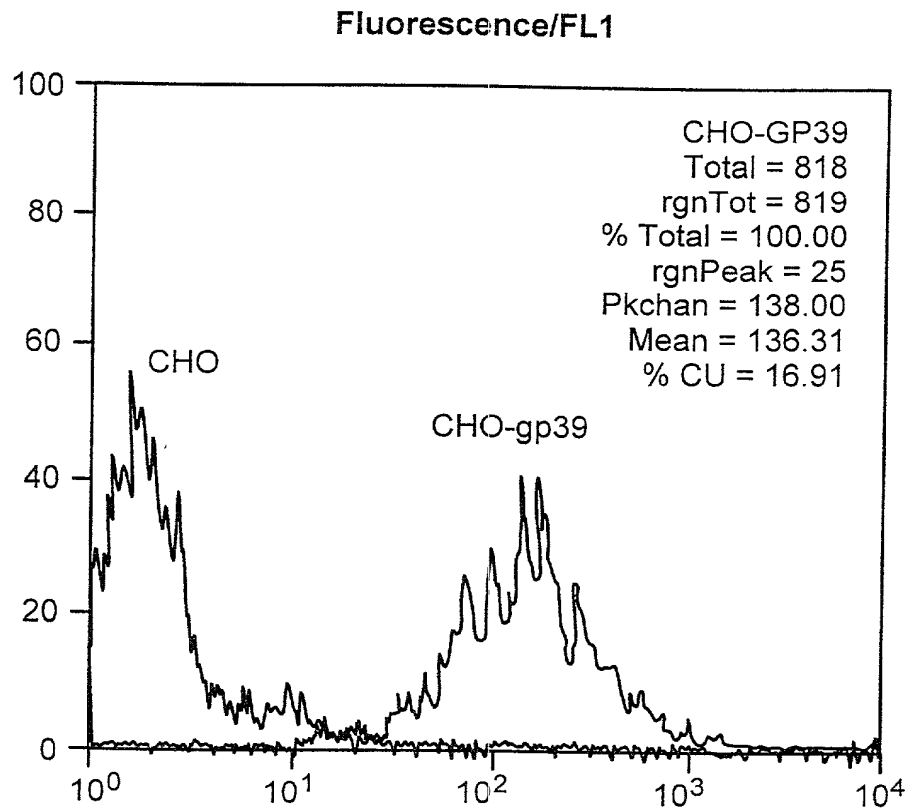
L = Leader

FIG. 2



Anti-gp39 inhibits B cell proliferation and differentiation, but not allogeneic T cell proliferation. A. Human PBL were cultured in 96 well plates (0.1×10^6 /well) in the presence or absence of the 20% (v/v) soluble gp39-CD8 (sgp39-CD8) fusion protein and 5 ug/ml rhIL-4 for 3 d. Anti-gp39 mAb, 24-31 (●), or a control murine IgG1 mAb (○), were added at a range of concentrations (1.25-10 ug/ml). Cultures were pulsed with 1 μ Ci 3 H-thymidine during the final 6 hr of a 72 hr culture period. B. Mitomycin treated T cells (5×10^4 /well) activated with immobilized anti-CD3 (64.1) were cultured with 2.5×10^4 /well IgD⁺ B cells in 96-well microtiter plates for 12d in the presence or absence of various concentrations (0.1-10.0 ug/ml) of anti-gp39 mAb, 24-31. Culture supernatants were subsequently assayed for IgM (□), IgG (◆), and IgA (△) by isotype specific ELISA.

FIG. 3



FACS analysis of non-transfected CHO cells and a gp39 transfectant. 1×10^6 cells were treated with the mouse anti-gp39 antibody 24-31 and then with a goat-anti-mouse IgG-FITC conjugate (Southern Biotechnology Associates). The samples were analyzed on FACScan (Becton Dickinson).

24-31 Humanized V_L #1

5'	<u>BglIII</u>		9		18		27		36		45		54					
	AGA	TCT	CTC	ACC	ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC
					M	G	F	K	M	E	S	Q	F	L	A	F	V	F
	63		72		81		FR1		90		99		108					
	GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CCA
	A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	P
	117		126		135				144		153		CDR1		162			
	TCT	TTC	CTC	TCC	GCC	TCC	GTA	GGA	GAC	AGG	GTC	ACC	ATC	ACC	TGC	AAG	GCC	AGT
	S	F	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S
	171		180		189		FR2		198		207		216					
	CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	AAG	TCT	CCT
	Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	K	S	P
	225		234		CDR2		243		252		FR3		261		270			
	AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC
	K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F
	279		288		297		306		315		324							
	TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	TCT	CTC	CAG	CCA
	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
	333		342		351		CDR3		360		369		378					
	GAA	GAC	TTC	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC
	E	D	F	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F
	FR4		387		396		405		<u>BsiWI</u>		3'							
	GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA	CGT	ACG							
	G	G	G	T	K	L	E	I	K	R	T							

FIGURE 4

24-31 Humanized V_L #2

5'	<u>BglIII</u>				9					18					27					36					45					54		
	AGA	TCT	CTC	ACC		ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC													
						M	G	F	K	M	E	S	Q	F	L	A	F	V	F													
					63					72					81					90	FR1					99					108	
	GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CCA														
	A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	P														
					117					126					135					144					153	CDR1					162	
	GAT	TCT	CTC	GCC	GTG	TCC	CTC	GGA	GAG	AGG	GCC	ACC	ATC	AAC	TGC	AAG	GCC	AGT														
	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	A	S														
					171					180					189	FR2					198					207					216	
	CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	CAA	TCT	CCT														
	Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	Q	S	P														
					225					234	CDR2					243					252	FR3					261					270
	AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC														
	K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F														
					279					288					297					306					315					324		
	TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	TCT	CTC	CAG	GCC														
	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	A														
					333					342					351	CDR3					360					369					378	
	GAA	GAC	GTG	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC														
	E	D	V	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F														
					FR4	387					396					405	<u>BsiWI</u>					CGT	ACG	3'								
	GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA																							
	G	G	G	T	K	L	E	I	K	R	T																					

FIGURE 5

24-31 Humanized V_H #1

5'	SalI		9			18			27			36			45			54		
	GTC	GAC	ATG	ATG	GTG	TTA	AGT	CTT	CTG	TAC	CTG	TTG	ACA	GCC	CTT	CCG	GGT	TTC		
			M	M	V	L	S	L	L	Y	L	L	T	A	L	P	G	F		
	CTG	TCA	63 FR1			72			81			90			99			108		
	GAG	GTG	CAG	CTT	CAG	GAG	TCA	GGA	CCT	GGC	CTC	GTG	AAA	CCT	TCT	GAG				
	L	S	E	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	E		
	ACT	CTG	117			126			135			144			153 CDR1			162		
	TCC	CTC	ACC	TGT	ACC	GTC	TCT	GGC	GAC	TCC	ATC	ACT	AAT	GGT	TTC	TGG				
	T	L	S	L	T	C	T	V	S	G	D	S	I	T	N	G	F	W		
	ATC	171 FR2			180			189			198			207 CDR2			216			
	TGG	ATC	CGG	AAA	CCA	CCA	GGG	AAT	AAA	CTT	GAG	TAC	ATG	GGC	TAC	ATA	AGT			
	I	W	I	R	K	P	P	G	N	K	L	E	Y	M	G	Y	I	S		
	TAC	AGT	225			234			243			252			261 FR3			270		
	GGT	AGC	ACT	TAC	TAC	AAT	CCA	TCT	CTC	AAG	AGT	CGA	ATC	TCC	ATC	TCT				
	Y	S	G	S	T	Y	Y	N	P	S	L	K	S	R	I	S	I	S		
	CGC	279			288			297			306			315			324			
	GAC	ACA	TCC	AAG	AAC	CAG	TTC	TCT	CTA	AAG	TTG	TCT	TCT	GTG	ACT	GCC	GCC			
	R	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A		
	GAC	ACA	333			342			351 CDR3			360			369			378		
	GGC	GTG	TAT	TAC	TGT	GCC	TGC	CGC	AGT	TAC	GGG	AGG	ACC	CCG	TAC	TAC				
	D	T	G	V	Y	Y	C	A	C	R	S	Y	G	R	T	P	Y	Y		
	TTT	GAC	387 FR4			396			405			414			423 NheI			3'		
	TTC	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACC	GTC	TCC	TCA	GCT	AGC						
	F	D	F	W	G	Q	G	T	T	L	T	V	S	S	A	S				

FIGURE 6

[illegible]

FIGURE 7

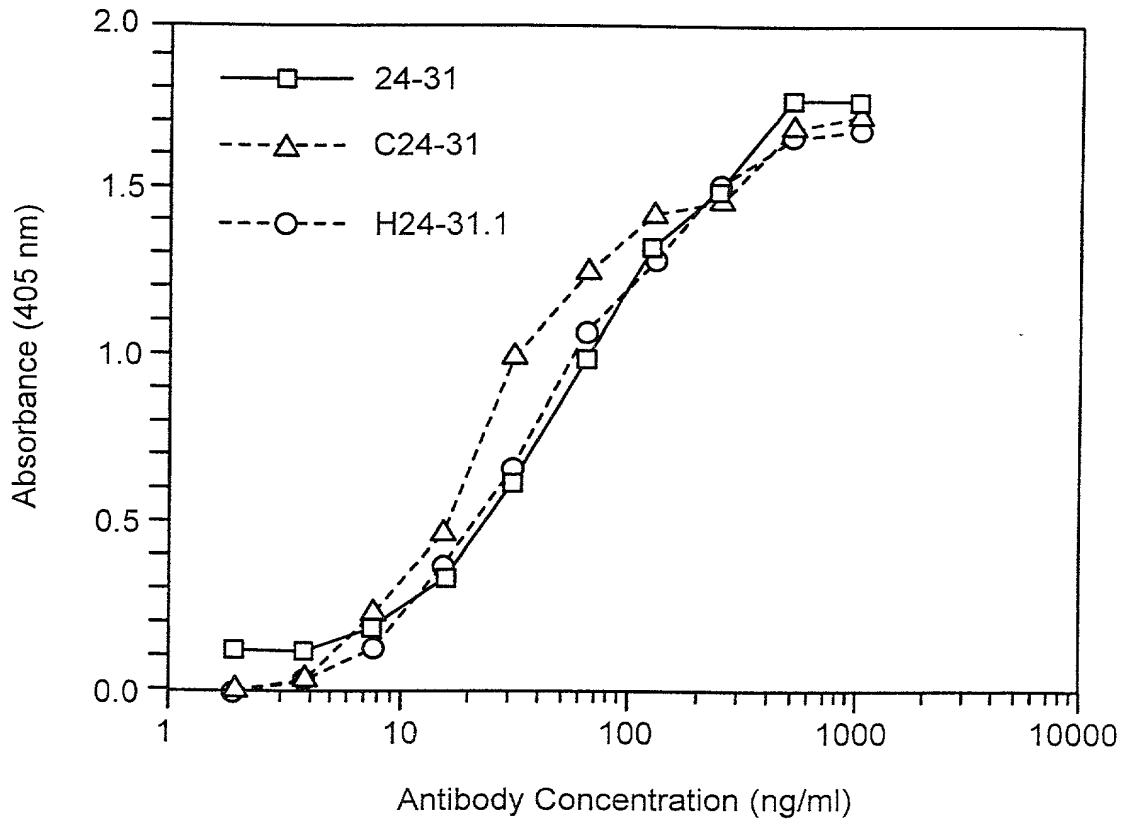
Anti gp39 24-31 V_H Sequence

SalI		9		18		27		36		45		54						
5'	GTC	GAC	ATG	ATG	GTG	TTA	AGT	CTT	CTG	TAC	CTG	TTG	ACA	GCC	CTT	CCG	GGT	TTC
			M	M	V	L	S	L	L	Y	L	L	T	A	L	P	G	F
			+1															
	CTG	TCA	GAG	GTG	CAG	CTT	CAG	GAG	TCA	GGA	CCT	AGC	CTC	GTG	AAA	CCT	TCT	CAG
	L	S	E	V	Q	L	Q	E	S	G	P	S	L	V	K	P	S	Q
			117			126			135			144			153	CDR1	162	
	ACT	CTG	TCC	CTC	ACC	TGT	TCT	GTC	ACT	GGC	GAC	TCC	ATC	ACT	AAT	GGT	TTC	TGG
	T	L	S	L	T	C	S	V	T	G	D	S	I	T	N	G	F	W
			171	FR2		180			189			198			207	CDR2	216	
	ATC	TGG	ATC	CGG	AAA	TTC	CCA	GGG	AAT	AAA	CTT	GAG	TAC	ATG	GGC	TAC	ATA	AGT
	I	W	I	R	K	F	P	G	N	K	L	E	Y	M	G	Y	I	S
			225			234			243			252			261	FR3	270	
	TAC	AGT	GGT	AGC	ACT	TAC	TAC	AAT	CCA	TCT	CTC	AAG	AGT	CGA	ATC	TCC	ATC	ACT
	Y	S	G	S	T	Y	Y	N	P	S	L	K	S	R	I	S	I	T
			279			288			297			306			315			324
	CGC	GAC	ACA	TCC	CAG	AAC	CAG	TTC	TAC	CTA	CAA	TTG	AAT	TCT	GTG	ACT	ACT	GAG
	R	D	T	S	Q	N	Q	F	Y	L	Q	L	N	S	V	T	T	E
			333			342			351	CDR3		360			369			378
	GAC	ACA	GGC	ACA	TAT	TAC	TGT	GCC	TGC	CGC	AGT	TAC	GGG	AGG	ACC	CCG	TAC	TAC
	D	T	G	T	Y	Y	C	A	C	R	S	Y	G	R	T	P	Y	Y
			387	FR4		396			405			414			423	NheI		
	TTT	GAC	TTC	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACC	GTC	TCC	TCA	GCT	AGC	3'	
	F	D	F	W	G	Q	G	T	T	L	T	V	S	S	A	S		

FIGURE 8

FIG. 9

Direct Binding of Anti-gp39 Antibodies to mgp39 CHO Cells

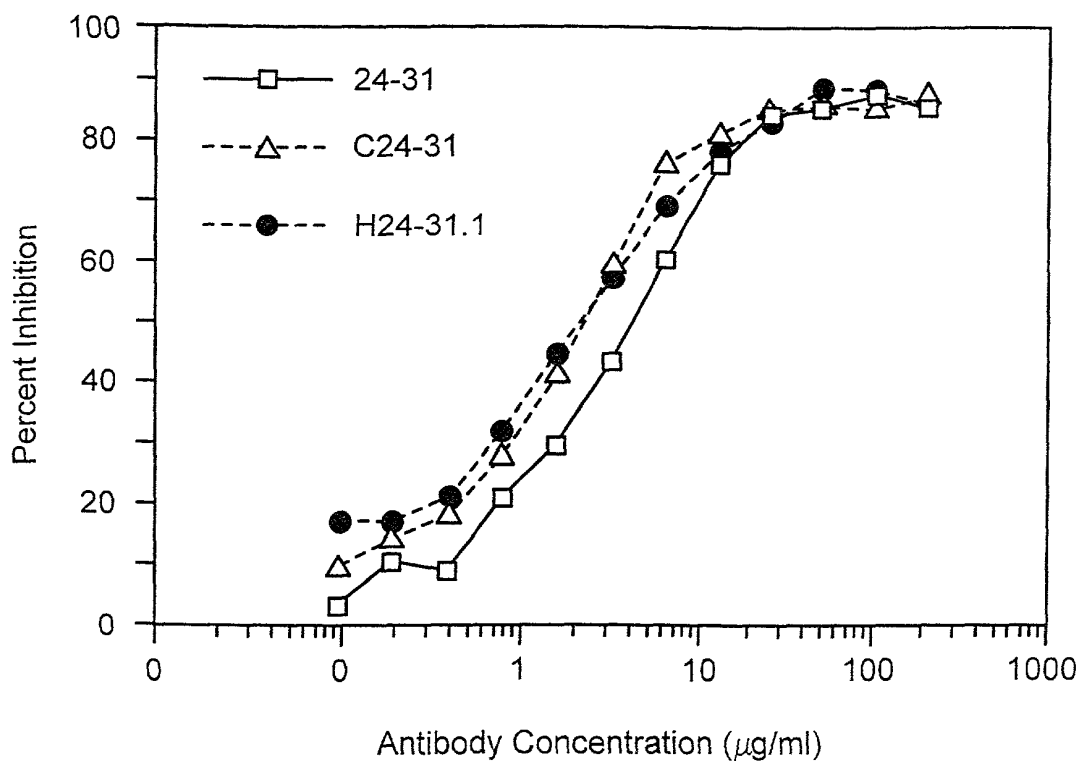


Fifty μ l of 1 μ g/ml solution of each anti-gp39 antibody (murine, chimeric and humanized version 1 of 24-31) was added to wells containing poly-l-lysine fixed mgp39 CHO cells. After a 2 hour incubation, the bound antibodies were detected with either goat anti-human IgG HRP or goat anti-mouse IgG HRP. The binding capacity of each antibody was compared on a plot of absorbance vs antibody concentration.

The figure shows that the half maximal binding in ELISA is achieved at similar concentrations for all three versions, at approximately 40 ng/ml.

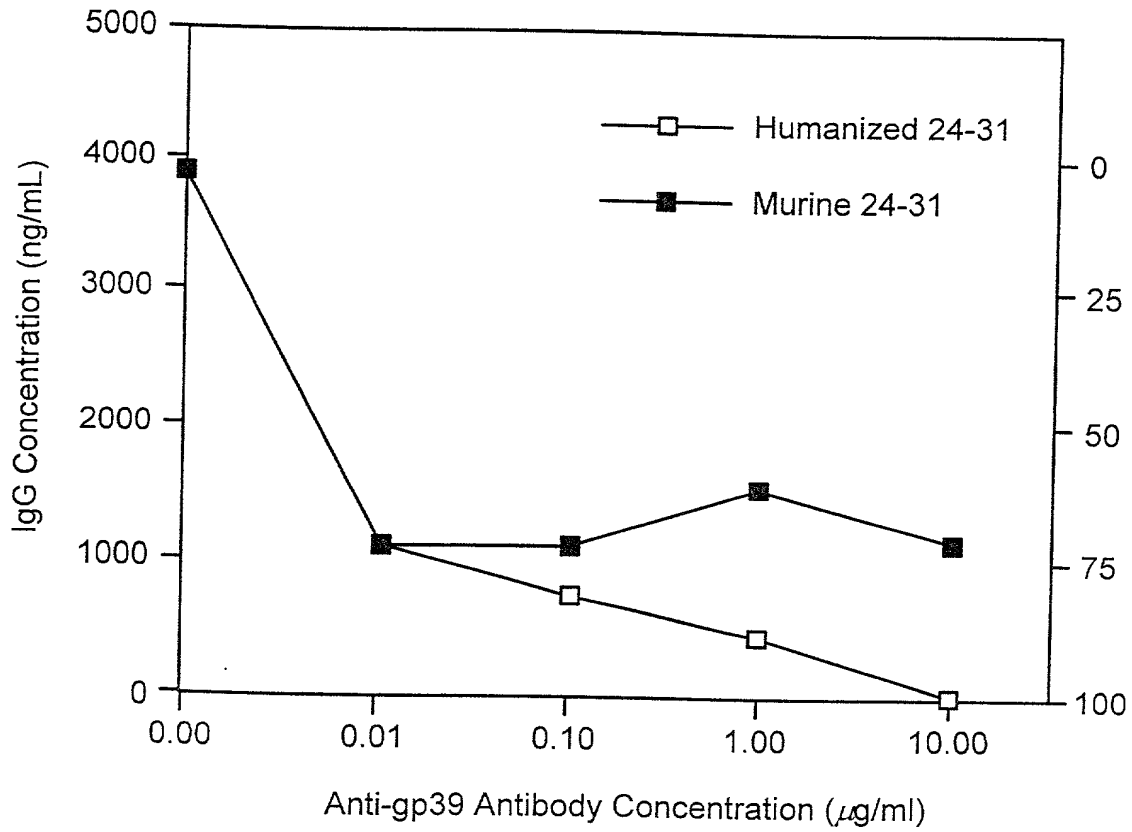
FIG. 10

**Competition Binding of 200 ng/ml Mouse Anti-gp39 Biotin
with Anti-gp39 Antibodies on mgp39 CHO Cells**



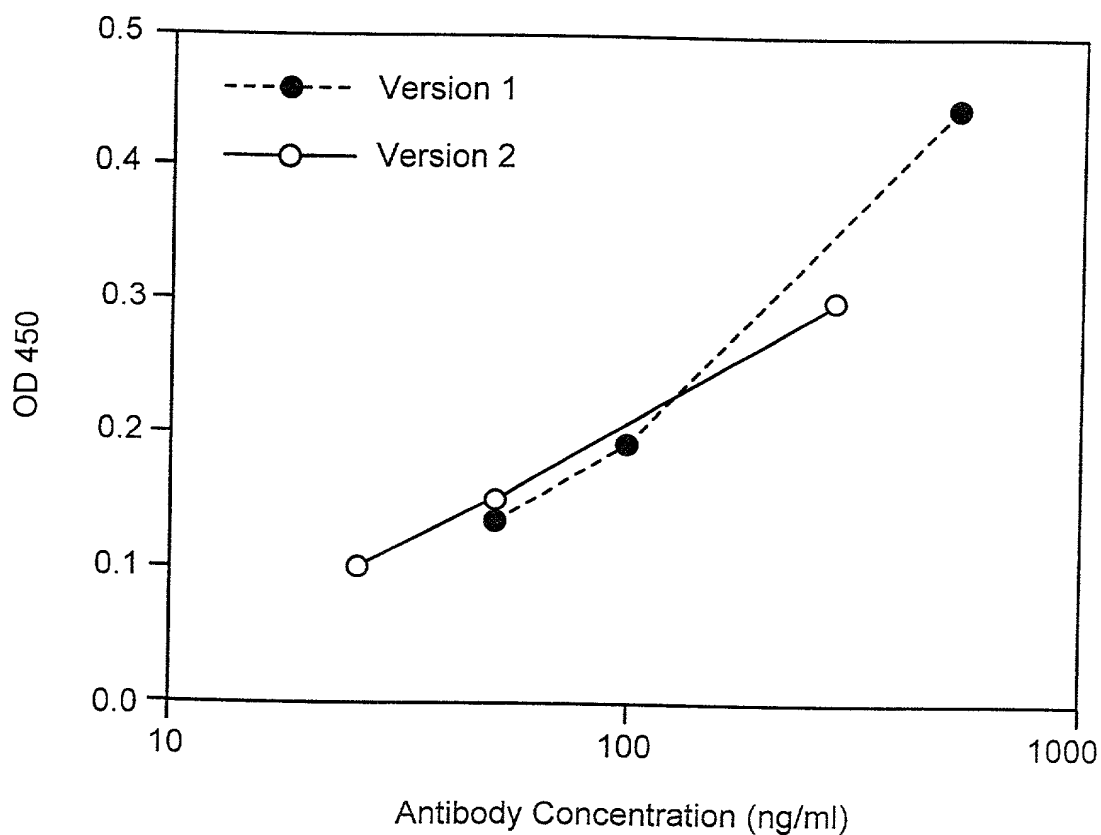
The figure shows that competition for binding to gp39 between biotin labeled 24-31 and the mouse, chimeric and humanized versions 1 are comparable, possibly with the humanized version slightly better than the original antibody, with half-maximal competition at 2 and 4 $\mu\text{g/ml}$, respectively.

FIG. 11



Purified, mitomycin C treated T cells were added into cell culture plates coated with anti-CD3 antibody. Autologous purified B cells were mixed with antibody at described concentrations and added to these plates in regular growth media. After 10 days the supernatant was tested for content of human IgM.

FIG. 12



CHO cell supernatant containing humanized 24-31 version 1 and version 2 in unknown amounts, was incubated on mgp39-CHO cells for 2 hours. After a wash, the amount of bound antibody was determined. The same supernatants were tested in parallel on an ELISA plate coated with Goat α Human γ , to determine the concentration of human IgG present relative to a control of known concentration. The binding data were normalized relative to the total antibody concentration.

FIG. 13

Scatchard Analysis

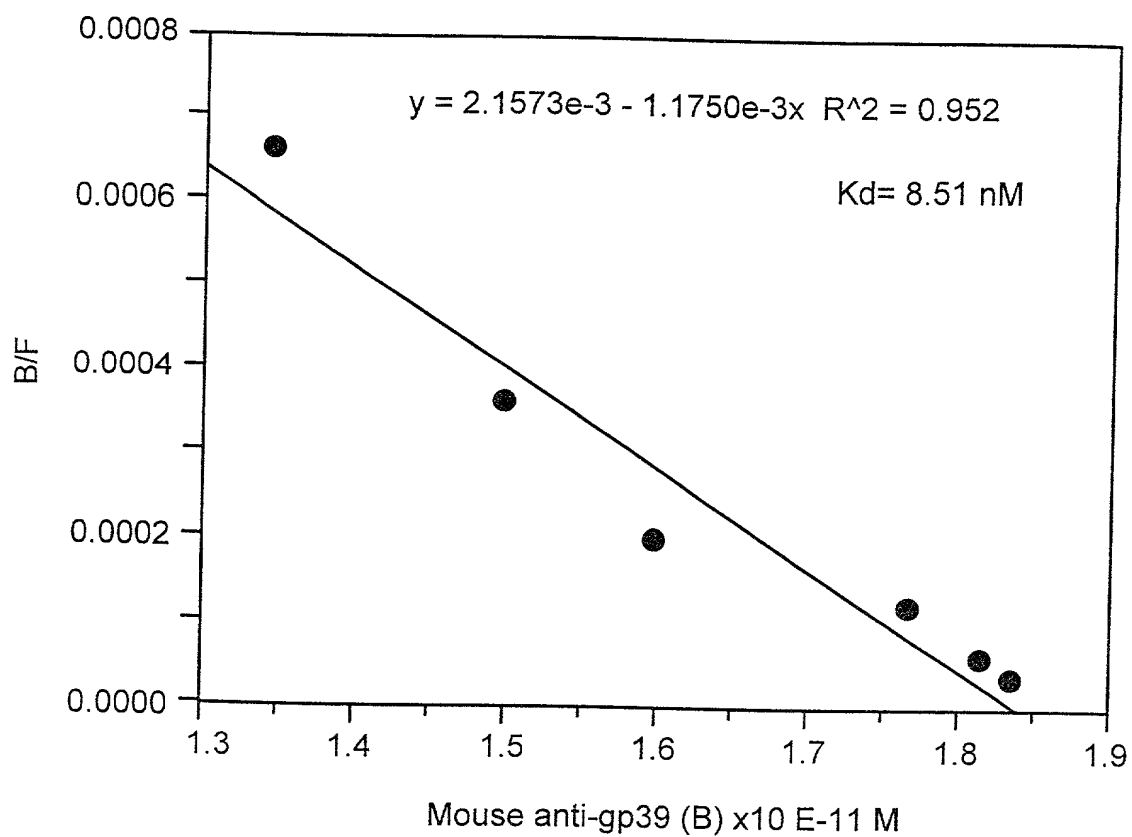


FIG. 14

Scatchard Analysis

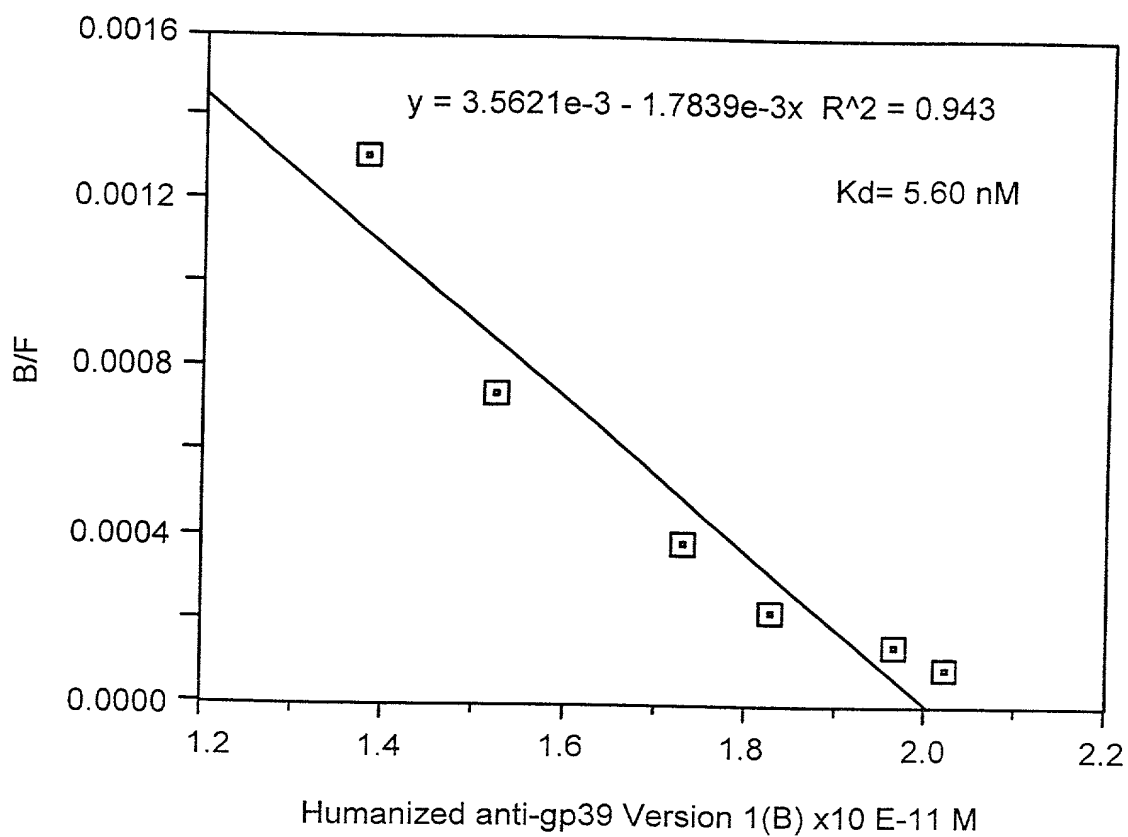
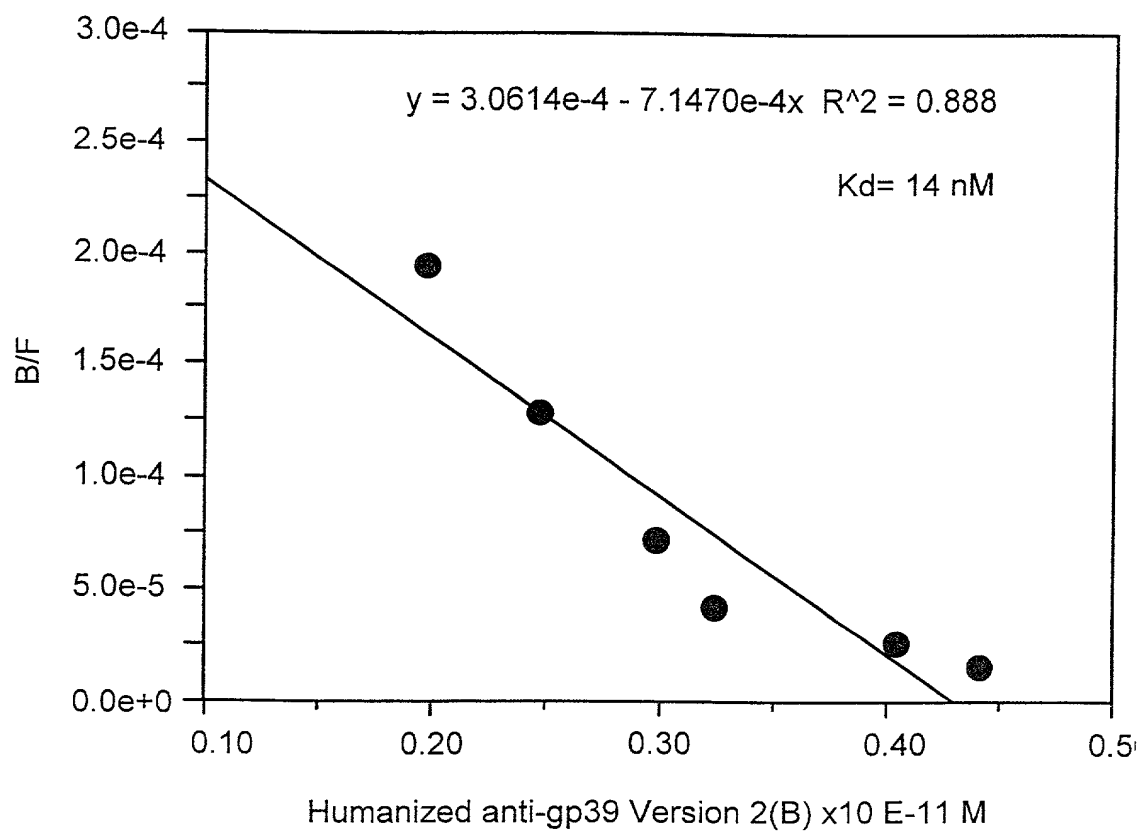


FIG. 15

Scatchard Analysis



Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-2 Cytokine Release from CD4+ T Cells in Presence of Immobilized Anti-CD3

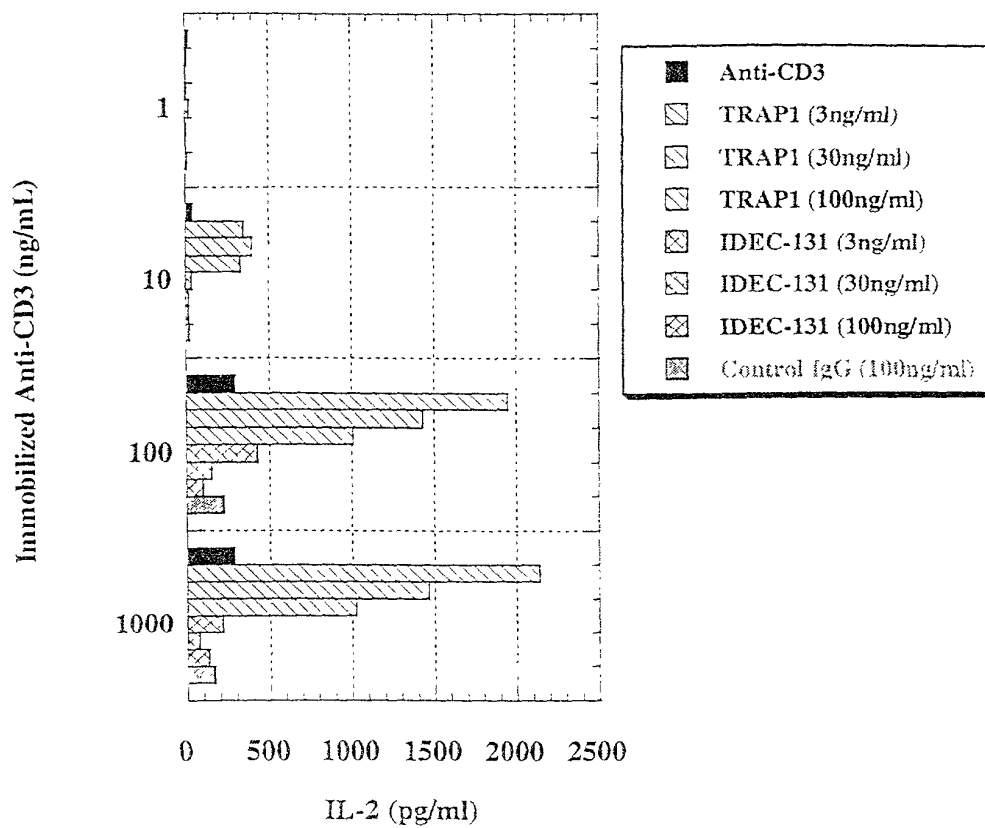


Figure 16

Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-4 Cytokine Release from CD4+ T cells in Presence of Immobilized Anti-CD3

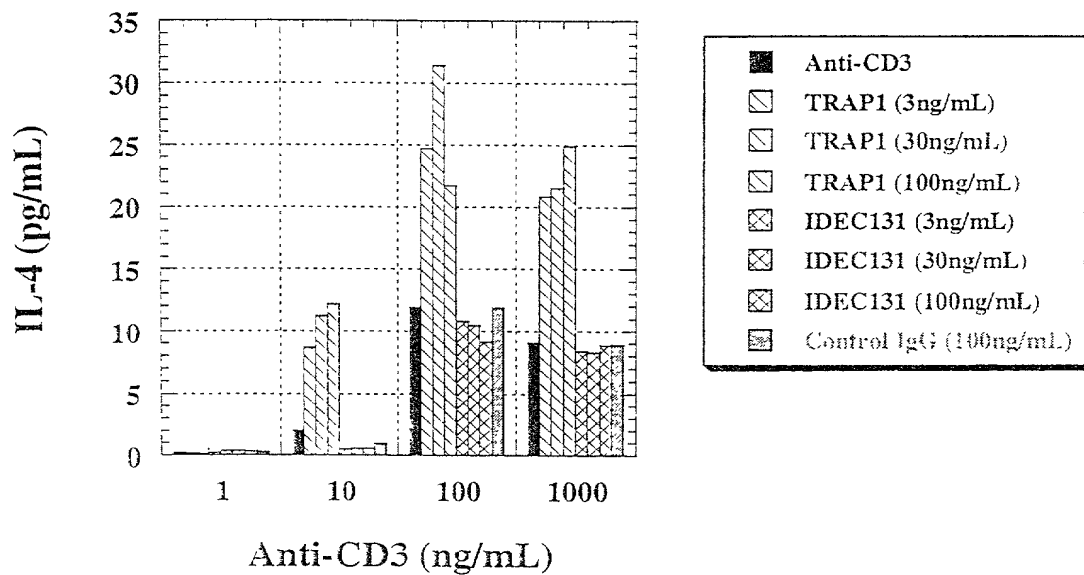


Figure 17

Stimulation of IL-2 by Agonist TRAP1 Anti-CD40L is Dependent on Co-stimulation with Anti-CD3 and Signaling Through CD40L

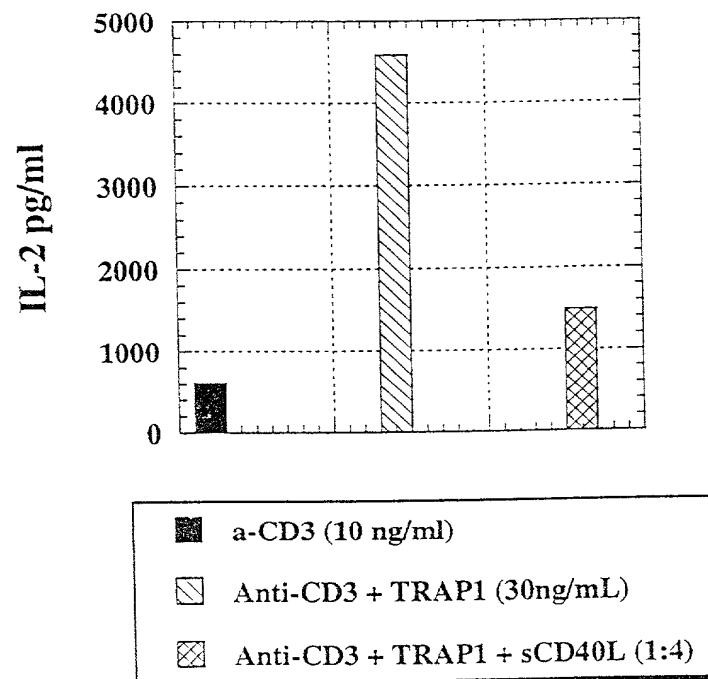
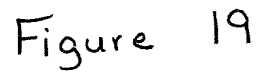


Figure 18

[illegible]

Thymidine Uptake (C/M)

